

Additional File 6

Phylogenetic trees of the fast evolving genes and of their putative orthologues and paralogues .

We used phylogenetic tree analysis to test the possibility that the rates of evolution observed in genes evolving fast along a branch of the phylogeny and in the candidate genes for positive selection were instead the result of a mis-identification of the putative orthologues.

For each candidate gene detected at a false discovery rate threshold of 0.20 (see Table XX), we searched the homologues (and possibly paralogues) in *A. thaliana* and in the two *Cardamine* datasets. BLAST searches identified putative paralogous of six of such genes in at least one among *A. thaliana*, *C. resedifolia* and *C. impatiens*. For these genes we proceeded with the phylogenetic analysis by first searching for the homologues of the *A. thaliana* gene (and, if present, of its paralogues) in other eudicots using BLASTx. Then, sequences of BLAST hits with a reasonable e-value ($< 10^{-50}$) were aligned using MUSCLE [1], and the subsequent alignment was used to build an unrooted neighbor joining phylogenetic tree using PAUP* [2] (bootstrap values obtained from 1,000 replications). Trees were drawn using Geneious 5.4 [3].

Phylogenetic trees were obtained for the candidate genes AT1G49750 (panel A), AT1G54040 (panel B), AT1G71040 (panel C), AT2G31610 (panel D), AT4G17520 (panel E), and AT5G62680 (panel F).

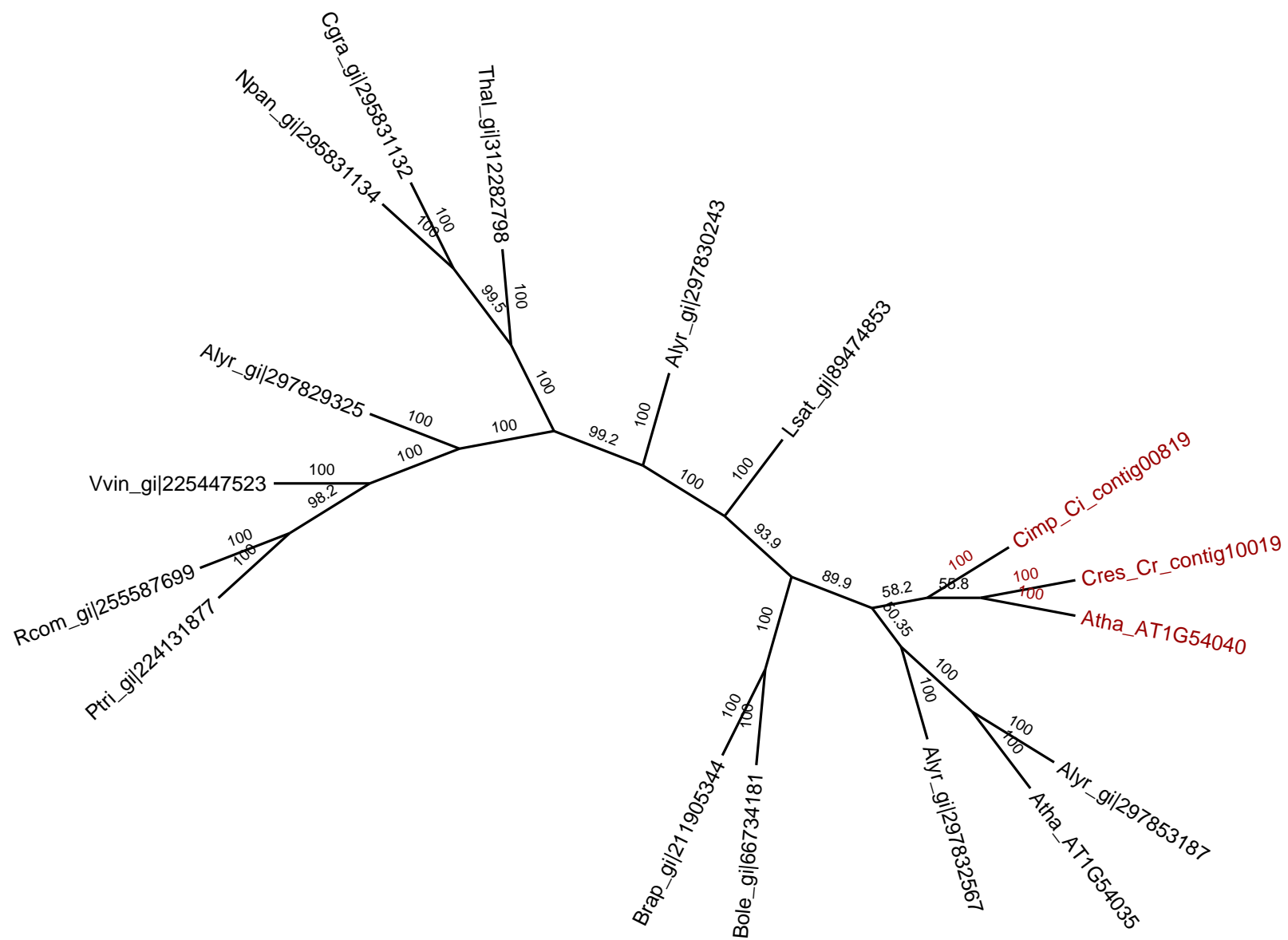
Sequences of the *A. thaliana*, *C. impatiens* and *C. resedifolia* orthologues used in our analyses are highlighted in red. Sequences were named using a four letter code identifying the species (see legend below), and followed by either the AGI name of the gene (for *A. thaliana*), the name of the contig (for *C. impatiens* and *C. resedifolia*), or the unique NCBI gene identifier.

Alyr = *Arabidopsis lyrata*; **Atha** = *Arabidopsis thaliana*; **Bole** = *Brassica oleracea*; **Brap** = *Brassica rapa*; **Cgra** = *Capsella grandiflora*; **Cimp** = *Cardamine impatiens*; **Cres** = *Cardamine resedifolia*; **Ghir** = *Gossypium hirsutum*; **Gmax** = *Glycine max*; **Lsat** = *Lepidium sativum*; **Mtru** = *Medicago truncatula*; **Npan** = *Neslia paniculata*; **Ptri** = *Populus trichocarpa*; **Rcom** = *Ricinus communis*; **Stub** = *Solanum tuberosum*; **Thal** = *Thellungiella halophila*; **Vvin** = *Vitis vinifera*.

References

1. Edgar RC: **MUSCLE: multiple sequence alignment with high accuracy and high throughput**. Nucleic Acids Res 2004, **32**:1792-1797.
2. Swofford DL: **PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4**. Sinauer Associates, Sunderland, Massachusetts; 2003.
3. Drummond AJ, Ashton B, Buxton S, Cheung M, Cooper A, Duran C, Field M, Heled J, Kearse M, Markowitz S, Moir R, Stones-Havas S, Sturrock S, Thierer T, Wilson A. **Geneious v5.4**, Available from <http://www.geneious.com>

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